A new species of *Cuthona* Alder and Hancock, 1855 (Gastropoda: Heterobranchia: Nudibranchia: Tergipedidae) from the Caribbean Sea

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ABSTRACT

Tergipedid nudibranch specimens from the Caribbean Sea previously identified as *Cuthona caerulea* are here described as a new species. Sequence data for the mitochondrial CO1 and 16S genes as well as the nuclear H3 are provided. A preliminary molecular phylogeny including other *Cuthona* species available in GenBank produced inconclusive results, but the new species is morphologically distinct from European specimens of *Cuthona caerulea*. Differences include radular teeth and reproductive morphology, as well as the external coloration.

INTRODUCTION

Cuthona Alder and Hancock, 1855 is a group of tergipedid nudibranchs characterized by having crowded rows of cerata, a broad foot, and arch-shaped radular teeth (Miller, 1977). Species of *Cuthona* feed on hydroids, and are most diverse in tropical and subtropical regions (Gosliner, 1981). *Cuthona* is taxonomically complex, and its taxonomic placement in relation to other genera in the Tergipedidae is controversial (Williams and Gosliner, 1979; Gosliner and Griffiths 1981; Miller; 1977; Brown, 1980; Miller, 2004).

Cuthona caerulea is a northeastern Atlantic species characterized by having a white body with numerous cerata with blue (or green) and yellow (or orange) pigment. The coloration of this species is extremely variable, but specimens with distinct color patterns are morphologically similar and regarded as members of the same species (Thompson and Brown, 1984).

Thompson and Brown (1984) reported this species for the first time from the western Atlantic, based on specimens collected from Florida, as well as records from São Paulo, Brazil (based on a personal communication by Ev. Marcus). Later, another specimen from Florida was illustrated by Valdés et al. (2006). The western Atlantic animals display some differences in color pattern in comparison to the European ones, and some authors considered the former to belong to an undescribed species (Picton and Morrow, 1994; Calado, 2002).

In this paper we examined additional specimens recently collected in Bocas del Toro, Panama, which are externally similar to the animals illustrated from Florida by Valdés et al. (2006). These animals were found to be distinct from *C. caerulea* and are herein described as a new species.

MATERIALS AND METHODS

Specimen Collection: Four specimens were collected on unidentified hydroids at 1 m depth in Crawl Cay, Bocas del Toro, Panama, on July 30, 2015. Two specimens were preserved in ethanol 95% and two in RNAlater. The type material is deposited at the Museo de Malacología, Universidad de Panamá (MUMAUP) and the Natural History Museum of Los Angeles County (LACM).

Morphological Examination: One specimen (paratype) was dissected. The buccal mass was extracted and placed in a small glass container with NaOH 10% water solution for 60 min until the tissue was soft. The jaws were then removed and placed in ultrapure water for 5 min. The radula and remaining tissue was left for another 24 hrs. After this period, the radula was also removed from the NaOH solution and placed in ultrapure water for 5 min. The radula and jaws were mounted on a stub for scanning electron microscope (SEM) examination. The stub with the samples was coated with an Emitech K550x sputter coater at the Natural History Museum of Los Angeles County. The samples were examined under a Jeol JSM-6010 variable pressure SEM at the California State Polytechnic University.

The reproductive system was dissected from the paratype (LACM 3335), examined under a dissecting microscope (Nikon SMZ-100), and drawn with the aid of a *camera lucida* attachment. The penis was removed from the rest of the reproductive system and placed in 1 mL of hexamethyldisilazane until all the liquid evaporated. The dry and hardened penis was then mounted on a stub and sputter coated for SEM examination.

DNA Amplification and Sequencing: DNA from the paratype was sequenced for this study. DNA extractions were performed using approximately 1–3 mg of tissue taken from the foot of the animal, followed by a hot Chelex® extraction protocol with minor modifications. The tissue sample was placed into a 1.7-mL tube containing 1.0 mL TE buffer (10 mM Tris, 1 mM EDTA, pH 7.8) and incubated overnight at room temperature in a rotator. The sample was centrifuged for 3 min at 21,130 g. Subsequently, 975 µL of the original 1 mL of TE buffer was removed without disturbing the pellet of tissue. Then, 175 µL of Chelex® solution was added and heated in a 56°C water bath for 20 min and placed in a 100°C heating block for 8 min. The supernatant was the final product used for the polymerase chain reaction (PCR).

PCR was used to amplify portions of the mitochondrial cytochrome c oxidase 1 (CO1) and 16S ribosomal RNA (16S) genes, as well as the nuclear histone 3 (H3) gene. The following universal primers were used to amplify the fragments of interest: CO1 (LCO1490 5′-GGTCAAC AAATCATAAAGATATTGG-3′, HCO2198 5′TAAACTT CAGGGTGACCAAAAAATCA-3′ developed by Folmer et al., 1994), 16S rRNA (16S ar-L 5′-CGCCTGTTTAT CAAAAACAT-3′, 16S br-H 5′-CCGGTCTGAACTCAG ATCACGT-3′ developed by Palumbi, 1996) and H3 (H3 AF 5′-ATGGCTCGTACCAAGCAGACGGC-3′, H3 AR 5′-ATATCCTTGGGCATGATGGTGAC-3′ developed by Colgan et al., 1998). Confirmation of amplification was carried out using agarose gel electrophoresis with ethidium bromide to detect the presence of DNA. PCR products were sent to Source BioScience (Santa Fe Springs, California, USA) for sequencing. Sequences were assembled and edited using Geneious Pro R8 (http://www.geneious.com, Kearse et al. 2012). The sequences obtained were deposited in GenBank, under the accession numbers presented in Table 1.

Phylogenetic Analyses: Phylogenetic analyses were run with the new sequences obtained and a data set of other species of *Cuthona* compiled from GenBank (Table 1). Phylogenetic analyses were conducted for all genes concatenated and each gene individually. The best-fit models of evolution (GTR + G for CO1, HKY + G for 16S, HKY for H3, and GTR + I for the entire concatenated data set) were determined using the Akaike information criterion (Akaike, 1974) implemented in jModelTest (Darriba et al., 2012). A Bayesian analysis was conducted with MrBayes 3.2 (Ronquist et al., 2012), partitioned by gene (unlinked). The Markov chain Monte Carlo analysis was run with two runs of six chains for 10 million generations, with sampling every 100 generations. The default 25% burn-in was applied before constructing the majority-rule consensus tree. Convergence was confirmed by eye

Table 1. Sequences used in the phylogenetic analyses including species name, locality and GenBank accession numbers.

Species	Locality	COI	16S	H3
Cuthona abronia	California, USA	JQ699569	JQ699478	JQ699390
Cuthona caerulea	North Sea	AF249807	-	-
Cuthona cocoachroma	Washington, USA	GQ292071	-	-
Cuthona columbiana	Canada	KF643448	-	-
Cuthona concinna	Washington, USA	GQ292072	-	-
Cuthona divae	California, USA	JQ699569	JQ699479	JQ699391
Cuthona fulgens	California, USA	-	JQ699480	JQ699392
Cuthona lagunae	California, USA	-	JQ699481	JQ699393
Cuthona ocellata	Portugal	AY345043	-	-
Cuthona sibogae	-	-	GU550049	-
Cuthona sp. 1	Antarctica	GQ292068	-	-
Cuthona sp. 2	Antarctica	GQ292078	-	-
Cuthona sp. 3	Antarctica	GQ292066	-	-
Cuthona sp. 4	Antarctica	GQ292069	-	-
Cuthona sp. 5	Antarctica	GQ292067	-	-
Cuthona sp. 6	Antarctica	GQ292070	-	-
Cuthona sp. 7	Washington, USA	GQ292074	-	-
Cuthona sp. 8	Washington, USA	GQ292073	-	-
Cuthona sp. 9	Antarctica	GQ292075	-	-
Cuthona sp. 35	Philippines	JQ997026	JQ996820	JQ996921
Cuthona sp. PW-2014	French Polynesia	KJ522457	-	-
Cuthona sp. A	Philippines	JQ997019	JQ996814	JQ996913
Cuthona luciae	Panama	KX077954	KX077953	KX077955
Tergipes tergipes	Maine, USA	KJ434077	KJ434064	KJ434095

using the "Tracc" function in Tracer 1.5 (Rambaut and Drummond, 2007). Maximum likelihood analyses were conducted for the entire concatenated alignment with raxmlGUI 1.0 (Silvestro and Michalak, 2012) using the bootstrap + consensus option (10,000 replicates) and the GTR + I model.

RESULTS

The Bayesian consensus tree was relatively well-resolved, but most nodes were not supported in the maximum likelihood tree (Figure 1). Only two clades, one including *Cuthona fulgens* (MacFarland, 1966) from California and two unidentified species from Washington and the Philippines, and another including *Cuthona divae* (Er. Marcus, 1961) and *Cuthona concinna* (Alder and Hancock, 1843), are well supported. Additionally, the phylogenentic position of the specimens from Panama, sequenced here in relation to a specimen of *Cuthona caerulea* from Europe, was not resolved.

Anatomical data revealed consistent differences between Panamanian and European specimens. Therefore, the taxon from Panama is below described as a new species. The morphological differences are described in the Discussion section.

SYSTEMATICS

Tergipedidae Bergh, 1889

Cuthona luciae new species
(Figures 2–11)

Cuthona caerulea (*non* Montagu, 1804).—Thompson and Brown, 1984: 121; Valdés et al. 2006: 264, 265

External Morphology: Live animals up to 12 mm length. Body narrow, elongated (Figure 2). Cerata elongated, cylindrical, dorso-lateral, arranged in 13–14 vertical rows, with 4–5 cerata in each row. Oral tentacles smooth. Rhinophores smooth, similar in length to oral tentacles. Reproductive opening located on right side of body, between first and second groups of cerata. Anus acleiproctic, dorso-lateral, posterior to pericardium. Body background color gray with irregular yellow spots. Posterior of dorsum dark blue. Dense yellow spotting on pericardium, behind second row of cerata. Rhinophores

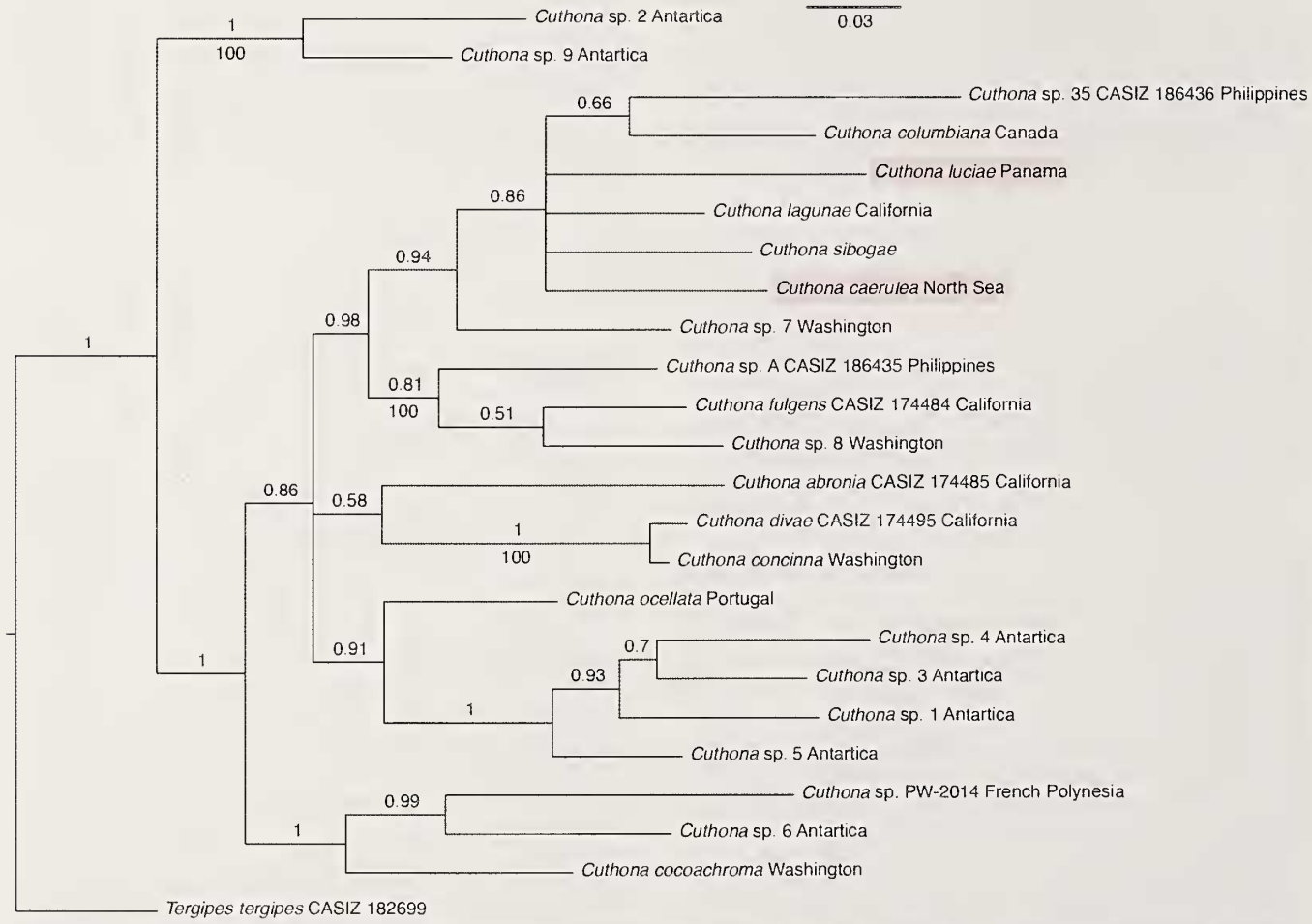
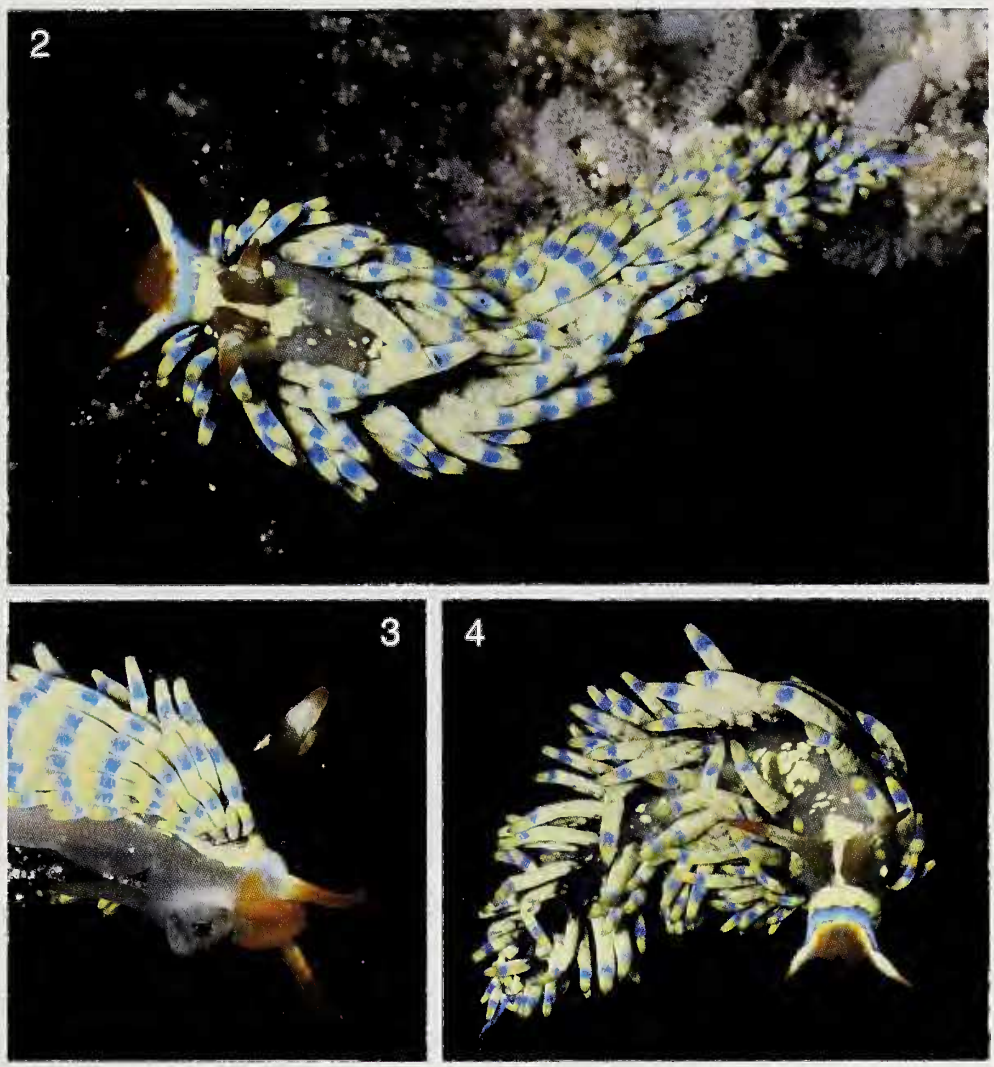


Figure 1. *Cuthona*. Bayesian consensus tree of the concatenated analysis including posterior probabilities and bootstrap values from the maximum-likelihood analysis.



Figures 2–4. *Cuthona luciae* new species. Photographs of live holotype (MUMAUP MOL-GAS-001). **2.** Dorsal view of animal on its hydroid prey, with egg mass visible. **3.** Lateral view of head. **4.** Dorsal view on black background.

opaque gray with white mid-region and orange apices. Yellow pigment on head, surrounding base of rhinophores. Cerata opaque yellow with blue band toward distal end before reverting to yellow. Oral tentacles opaque yellow with dark orange tips. Blue transverse band connecting bases of oral tentacles. Anterior end of head dark orange.

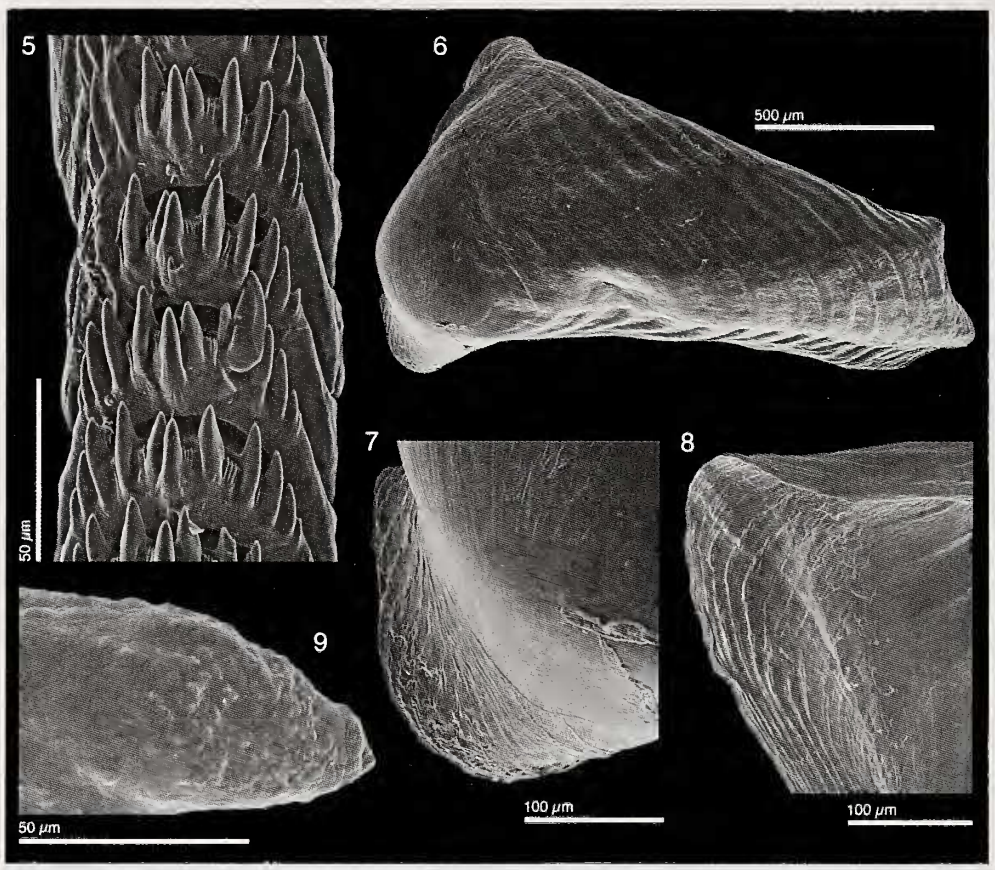
Anatomy: Radular formula 68 × 0.1.0 in 12 mm preserved length paratype (LACM 3335). Radular teeth with 9–10 large, sharp denticles, which decrease in size toward lateral sides of teeth and again toward center (Figure 5). Denticles separated by gaps, which become wider towards center of teeth. Gaps filled with tiny, sharp denticles, which vary in number depending on width of gap and are absent from most lateral gaps. Cusp about same length, or shorter, than central denticles, and only distinguishable from denticles because it emerges from slightly higher plane. Jaws elongate (Figure 6) with smooth masticatory borders (Figures 7–8).

Reproductive system (Figure 10) with an elongate ampulla connecting directly into female gland complex. Prostate emerges from female gland complex, near insertion point of ampulla. Prostate long and convoluted, narrowing abruptly at distal end, to expand again into deferent duct. Distal portion of deferent duct containing large penis with apical stylet (Figures 9, 11). Vagina slightly curved, connecting directly into rounded bursa copulatix.

Type Material: HOLOTYPE: MUMAUP MOL-GAS-001, July 30, 2015; PARATYPE: LACM 3335, July 30, 2015; all from type locality.

Type Locality: Crawl Cay, Bocas del Toro, Panama.

Geographic Range: Florida (Thompson and Brown 1984, Valdés et al. 2006) to Panama (present paper) and possibly Brazil (Thompson and Brown 1984).



Figures 5–9. *Cuthona luciae* new species. Scanning electron micrographs of radular teeth, jaws, and penis of paratype (LACM 3335). **5.** Radular teeth. **6.** Jaw. **7.** Dorsal view of the masticatory border. **8.** Ventral view of the masticatory border. **9.** Penis.

Etymology: Named after Lucía Valdés, daughter of the senior author.

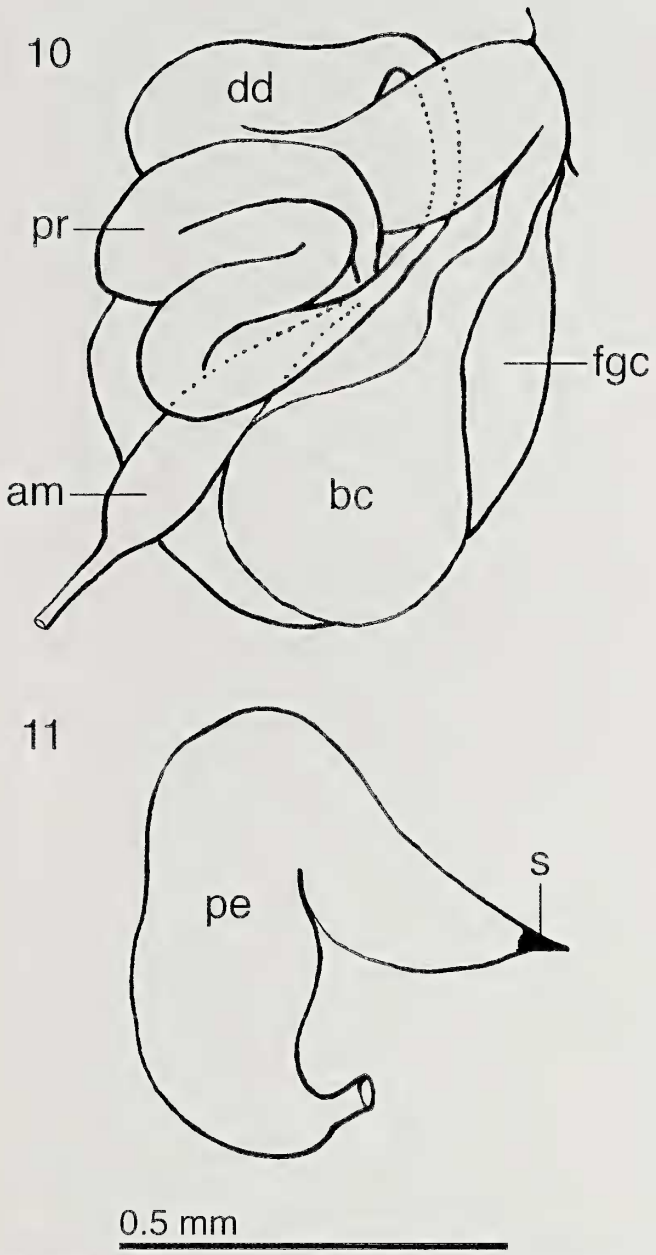
DISCUSSION

The phylogenetic analyses resulted in poorly supported trees. Although the Bayesian consensus tree contains well-supported nodes, many of those are not supported in the maximum likelihood consensus tree. This study has not produced a reliable phylogeny for the species of *Cuthona* sequenced to date. The results of this phylogenetic analysis are also inconclusive as to the position of the species here described relative to a European specimen of *Cuthona caerulea*. However, a Bast-n search in GenBank revealed that the COI sequence of *C. caerulea* from the North Sea in GenBank (AF249807) and the sequence from *C. luciae* are only 82% identieal, which is consistent with species-level differences. In addition, the morphological examinations revealed the presence of several unique characteristics that support that the Caribbean animals constitute a distinct species.

The radular teeth of *Cuthona luciae* are very different from those of *C. caerulea* described from European specimens. Schmekel and Portman (1982) illustrated three radular teeth in lateral view of a specimen collected in Naples, Italy. These teeth had 6 lateral denticles of similar size and a larger central cusp. Thompson and Brown (1984) illustrated one radular tooth of a specimen from Lundy, England, which had 5 lateral denticles, but was otherwise similar to the Mediterranean radula illustrated by Schmekel and Portman (1982). These radulae are very different from the Caribbean specimens here examined, in which the teeth contain denticles separated by gaps filled with tiny, sharp denticles, varying in number depending on the width of the gap. Additionally, the jaws of European specimens have a distinct masticatory border with denticles (Thompson and Brown, 1984), which is absent in the Caribbean animals, although Schmekel and Portman (1982) reported that it can be absent in Mediterranean specimens as well.

Schmekel and Portman (1982) illustrated the reproductive system of a specimen from Naples, Italy. Although the reproductive system of the specimen examined from the Caribbean is similar, there are two fundamental differences, the European specimens have a well-formed penial gland, absent in the Caribbean animal; in addition, the Caribbean animal has a penial stylet, which is not reported in the European specimen.

A similar species to *Cuthona luciae* is *Cuthona herrerai* Ortea, Moro, and Caballer, 2001, originally described from Cape Verde, Eastern Atlantic. The radular teeth of



Figures 10, 11. *Cuthona luciae* new species. Reproductive system of paratype (LACM 3335). **10.** Dorsal view of the reproductive system. **11.** Detail of the penis. Abbreviations: **am,** ampulla; **bc,** bursa copulatrix; **dd,** deferent duct; **fgc,** female gland complex; **pe,** penis; **pr,** prostate; **s,** penial stylet.

C. herrerai are very similar to those of *C. luciae* in having large denticles separated by gaps containing tiny denticles (Ortea et al. 2001). However, many other characteristics differentiate these two species, for example the jaws of *C. herrerai* contain denticles on the masticatory border, absent in *C. luciae*; *C. herrerai* has less rows of cerata and less cerata per row than *C. luciae*; more importantly, *C. herrerai* lacks orange pigment on the oral tentacles and the characteristic bright blue band on the head of *C. luciae*. Although the cerata of the two species bear similar colors, they are much brighter in *C. luciae*.

Also, *Cuthona iris* Edmunds and Just, 1983, originally described from Barbados, has a similar color pattern with yellow cerata, each with a blue band (see Valdés et al. [2006] for a color illustration). But the body of this species is predominantly yellow, including the rhinophores and oral tentacles, it has a light blue dorsal band, absent in *C. luciae*, and lacks the characteristic head pigmentation of *C. luciae*.

Although morphological evidence confirmed that *Cuthona luciae* is distinct from *C. caeurela* and other similar species such as *C. herrerai* and *C. iris*, further research is necessary to determine the phylogenetic position of *C. luciae* and to resolve the evolutionary relationships within Tergipedidae. The sequence data provided here should facilitate future work toward these goals.

ACKNOWLEDGMENTS

The SEM work was conducted at the California State Polytechnic University SEM laboratory supported by the US National Seience Foundation (NSF) grant DMR-1429674. The fieldwork in Panama was conducted during the course "Taxonomy and Biology of Sea slugs" funded by awards NSF DEB-1355190 and DEB-1355177, and organized by Rachel Collin of the Smithsonian Tropical Research Institute in Bocas del Toro. The travel expenses of AV and SB were also supported by the NSF, and those of VB by the British Ecological Society (travel grant 5585-6629), and the Bombay Natural History Society. Several staff members of the Smithsonian Tropical Research Institute in Bocas del Toro facilitated the fieldwork activities: Plinio Gondola, Urania Gonzalez, Tanyusha Grenald, Arcadio Castillo, Deyvis Gonzalez, and Nerea Nieto. Collecting of specimens was possible with permits ARAP 27 (2015) and ANAM SE/A-64-15 issued by the Panamanian Government.

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